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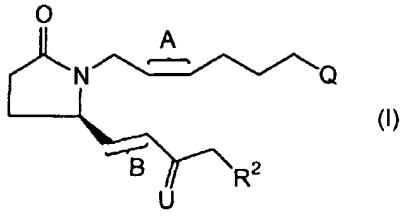
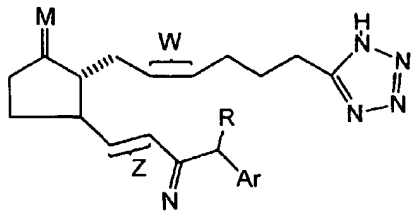
(54) Title: USE OF SELECTIVE EP4 RECEPTOR AGONISTS FOR THE TREATMENT OF LIVER FAILURE, LOSS OF PATENCY OF THE DUCTUS ARTERIOSUS, GLAUCOMA OR OCULAR HYPERTENSION



(I)



(II)

(57) Abstract: The present invention is directed to methods for treating liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension, comprising administering to the patient in need thereof a therapeutically effective amount of a selective EP_4 receptor agonist of formulae (I) or (II) wherein the variables A, B, Q, =U, and R^2 for Formula (I); and the variables Ar, =M, =N, R, W, and Z for Formula (II) are as defined in the specification.

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USE OF SELECTIVE EP_4 RECEPTOR AGONISTS FOR THE TREATMENT OF LIVER FAILURE, LOSS OF PATENCY OF THE DUCTUS ARTERIOSUS, GLAUCOMA OR OCULAR HYPERTENSION

FIELD OF THE INVENTION

The present invention relates to methods of using receptor selective prostaglandin (PGE_2) agonists for the treatment of liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension in animals, particularly mammals. More specifically, the present invention relates to such methods using type 4 (EP_4) receptor selective prostaglandin (PGE_2) agonists.

BACKGROUND OF THE INVENTION

The naturally occurring prostaglandins are comprised of several biological entities including PBD, PGE, PGF, PGG, PGH and PGI. It has been well documented that prostaglandins have effects on many of the organs and systems of the body. Prostaglandin E_2 (abbreviated as PGE_2 herein) is known to be a cyclooxygenase induced oxidative metabolite in the arachidonic acid cascade, and it has been well documented that prostaglandins, including PGE_2, have effects on many of the organs and systems of the body. For example, it is known that PGE_2 has cyto-protective activity, uterine contractile activity, a pain-inducing effect, a promoting effect on digestive peristalsis, an awakening effect, a sleep-inducing effect, a suppressive effect on gastric acid secretion, hypotensive activity and diuretic activity. In previous studies it has been found that the PGE_2 receptor has various subtypes, each possessing differing physiological roles. At this time, it is known that the PGE_2 receptor has four primary subtypes denoted EP_1, EP_2, EP_3 and EP_4, respectively, each of which mediates different effects in various tissues and cells (Coleman, R.A. et al., Pharm. Rev. **1994**, *46(2)*, 205-229). The EP_4 receptor is distributed in such organs as the thymus, heart, kidney, liver, intestine, womb, ductus arteriosus and bone, and it is known that the EP_4 receptor is related to relaxation of smooth muscle, differentiation and proliferation of lymphocytes, proliferation of mesangial cells, and collagen production of the fibroblasts. In both the pig and the dog, modulation of the EP_4 receptor has been characterized with

relaxation of the saphenous vein, and in the rabbit relaxation of the jugular vein occurs (Coleman, R.A. et al., Prostaglandins **1994**, *47*, 151).

Numerous studies have demonstrated the protective action of prostaglandin E_1 on experimental models of liver injury and on patients with fulminant viral hepatitis, with PGE_1 acting in many different ways to bring about this effect (Liu, X.L. et al. World J. Gastroenterol. **2000**, *6(3)*, 326-329). For example, PGE_1 could act upon the PGE_1 receptor of diseased vessels to dilate them and increase portal venous flow, improve the microcirculation of the liver, clear the metabolites of the liver cells and increase oxygen supply to the liver tissues.

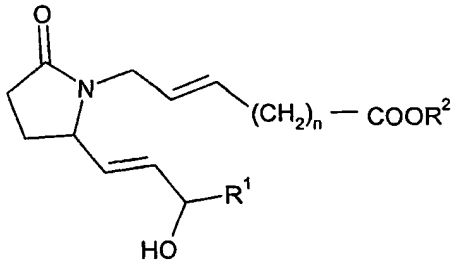
The EP_4 receptor is also expressed in the ductus arteriosus (Bhattacharya, M. et al., Circulation **1999**, *100*, 1751-1756). The ductus arteriosus is an arterial connection in the fetus, which directs blood away from the pulmonary circulation and towards the placenta where oxygenation occurs (Heymann, M.A.; Rudolph, A.M. Physiol. Rev. **1975**, *55*, 62-78). In one proposed model the EP_4 receptor in the ductus arteriosus acts as a sensor that responds to the perinatal drop in circulating levels of PGE_2 by triggering closure of the ductus arteriosus (Nguyen, M. et al., Nature **1997**, *390*, 78-81). Closure of the ductus arteriosus was observed in an *in vivo* fetal sheep model after administration of a selective EP_4 antagonist (PCT International Application WO 01/42281, published on June 14, 2001). Maintaining the ductus arteriosus in the open, or patent state is desirable in the fetus and in infants with certain types of congenital heart defects where pulmonary or systemic blood flow depends on patency of the ductus arteriosus. Maintaining patency of the ductus arteriosus in infants with certain other types of congenital heart disease such as coarctation of the aorta, transposition of the great arteries, and Ebstein's anomaly may also be desirable. For example, infants with coarctation of the aorta, a condition constituting 7% to 8% of congenital cardiac defects, may have sudden onset of heart failure, cardiovascular collapse, and severe metabolic acidosis as the ductus arteriosus closes and distal perfusion is compromised. In cases such as these, PGE_1 infusions have been utilized to reopen and maintain the patency of the ductus arteriosus prior to surgical repair of the defect.

An excess of aqueous humor in the anterior chamber of the eye can result in elevated intraocular pressure or ocular hypertension. Ocular hypertension is a symptom and/or risk factor for glaucoma, a disease that can damage the optic nerve and cause blindness. The EP_4 receptor has been found in ocular tissues

involved in the production of the aqueous humor, such as human ciliary epithelial cells and human ciliary muscle cells (Mukhopadhyay et al., Biochem. Pharmacol. **1997**, *53*, 1249-1255). Trabecular meshwork cells are known to be involved in the regulation of intraocular pressure (Clark et al., Investigative Opthalmology & Visual Science **1994**, *35*, 281-294; and Lutjen-Drescoll, Progress in Retinal and Eye Research **1998**, *18*, 91-119). The EP_4 receptor has also been found in human trabecular meshwork cells and it has been proposed that activation of the EP_4 receptors in the trabecular meshwork cells can result in relaxation of these cells, thereby lowering intraocular pressure (PCT International Patent Application WO 00/38667, published on July 6, 2000).

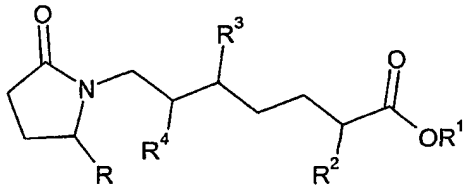
As PGE_1 and PGE_2 bind to all four of the PGE_2 receptor subtypes (EP_1, EP_2, EP_3, and EP_4), various physiological activities may result, some of which may be an undesired side effect due to the lack of selectivity in binding to the PGE_2 receptor subtypes. Severe side effects have been associated with PGE_2 treatment. W.S.S. Jee, W.S.S. and Ma, Y.F. Bone, **1997**, *21*, 297-304.

Great Britain Patent Specification 1 553 595 discloses compounds of the formula



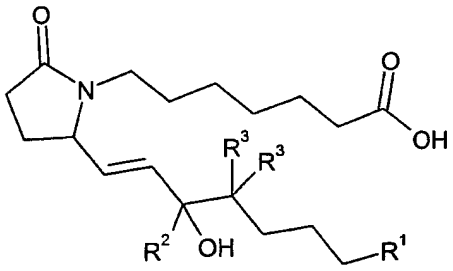
wherein the double bonds are cis or trans and the variables are defined as set forth therein. Those compounds are disclosed as having spasmogenic and spasmolytic activity, for example bronchodilatory and antihypertensive effects. The compounds are also disclosed as having utility in the inhibition of the secretion of gastric juice and as having abortive effects.

U.S. Patent No. 4,115,401 discloses compounds of the formula



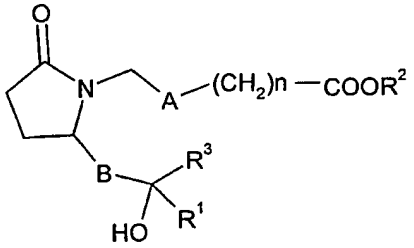
wherein the variables are defined as set forth therein. Those compounds are disclosed as having spasmogenic, cardiovascular and bronchodilatory effects.

U.S. Patent No. 4,113,873 discloses compounds of the formula



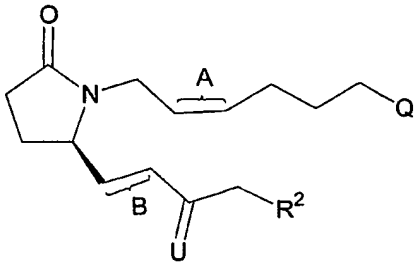
wherein the variables are defined as set forth therein. Those compounds are disclosed as having utility as a bronchodilator, as an antihypertensive agent, as an enhancer of spontaneous contraction of the uterus and for the treatment of gastro-intestinal disorders or gastric ulcers.

Great Britain Patent Specification 1 583 163 discloses compounds of the formula



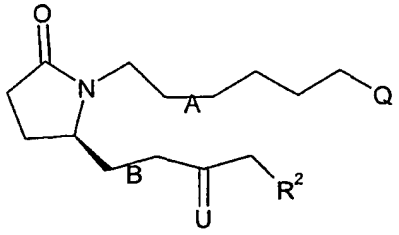
wherein the variables are defined as set forth therein. Those compounds are disclosed as having spasmogenic, bronchodilatory, vasoconstricting, vasodilating and abortive properties as well as utility in the inhibition of gastric acid secretion.

United States Patent No. 4,177,346, discloses compounds of the formula



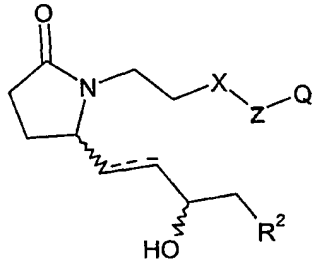
wherein the variables are defined as set forth therein. Those compounds are disclosed as having vasodilator, antihypertensive, bronchodilator, antifertility and antisecretory activity.

United States Patent Application Publication Nos. US 2001/0041729, which published on November 15, 2001, and US 2001/0047105, which published on November 29, 2001, disclose methods of treatment with compounds of the formula



wherein the variables are defined as set forth therein. The methods of treatment disclosed in US 2001/0041729 include the treatment of acute or chronic renal failure or dysfunction, or a condition caused thereby, such as hypertension, congestive heart failure, glomerulonephritis, uremia or chronic renal insufficiency. The methods of treatment disclosed in US 2001/0047105 include the treatment of conditions which present with low bone mass, particularly osteoporosis, frailty, an osteoporotic fracture, a bone defect, childhood idiopathic bone loss, alveolar bone loss, mandibular bone loss, bone fracture, osteotomy, bone loss associated with periodontitis, or prosthetic ingrowth.

United States Patent Application No. 09/990,556, which was filed on November 21, 2001, discloses compounds of the formula



wherein the variables are as defined therein. The compounds are useful for the treatment of conditions which present with low bone mass such as osteoporosis, frailty, an osteoporotic fracture, a bone defect, childhood idiopathic bone loss, alveolar bone loss, mandibular bone loss, bone fracture, osteotomy, bone loss associated with periodontis, prosthetic ingrowth, or kidney dysfunction.

U.S. Patent No. 3,932,389 provides 2-descarboxy-2-(tetrazol-5-yl)-11-desoxy-15-substituted-ω-pentanorprostaglandins with vasodilator activity, antihypertensive activity, bronchodilator activity, antifertility activity and antiulcer activity.

European Patent Application EP 1114816 discloses ω-substituted phenyl prostaglandin E derivatives useful for the treatment of immune diseases, asthma, abnormal bone formation, neurocyte death, pulmopathy, hepatopathy, sleeping disorders and platelet coagulations etc.

Certain 3,7-Dithiaprostanoic acid derivatives useful for treatment or prevention of immunologic diseases, asthma, abnormal bone formation, neuronal cell death, liver damage, nephritis, hypertension, myocardiac ischemia etc. are disclosed in U.S. Patent Nos. 5,892,099 and 6,043,275.

PCT International Patent Application No. WO 99/02164 discloses methods and compositions for treating impotence or erectile dysfunction using prostaglandins that are selective EP_2 or EP_4 prostanoid receptor agonists.

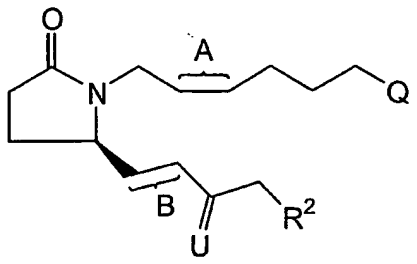
Certain EP_2 receptor agonists, useful as agents for lowering intraocular pressure, have been disclosed in U.S. Patent Nos. 5,462,968 and 5,698,598.

Certain prostaglandin E agonists useful for the treatment of glaucoma have been disclosed in PCT International Patent Application No. WO 00/38667, which published on July 6, 2000.

SUMMARY OF THE INVENTION

The present invention provides methods of treating liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension in a mammal comprising administering to said mammal a selective EP_4 receptor agonist, an isomer thereof, a prodrug of said agonist or isomer, or a pharmaceutically acceptable salt of said agonist, isomer or prodrug. The selective EP_4 receptor agonists useful in the methods of the present invention are 1,5-disubstituted-2-pyrrolidones of Formula I or 2-descarboxy-2-(tetrazol-5-yl)-11-desoxy-15-substituted-ω-pentanor-prostaglandins of Formula II. The 1,5-disubstituted-2-pyrrolidone compounds of Formula I can be prepared as disclosed in U.S. Patent No. 4,177,346, and U.S. Patent Application Publication US 2001/0047105, published on November 29, 2001. The preparation of 2-descarboxy-2-(tetrazol-5-yl)-11-desoxy-15-substituted-ω-pentanor-prostaglandins of Formula II is described in U.S. Patent No. 3,932,389.

A preferred group of the selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula I:



I

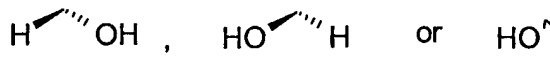
prodrugs thereof or pharmaceutically acceptable salts of said compounds or said prodrugs, wherein:

Q is $COOR^3$, $CONHR^4$ or tetrazol-5-yl;

A is a single or cis double bond;

B is a single or trans double bond;

=U is =O,



R^2 is α-thienyl, phenyl, phenoxy, monosubstituted phenyl or monosubstituted phenoxy, said substituents being selected from the group consisting of chloro, fluoro, phenyl, methoxy, trifluoromethyl and (C_1-C_3)alkyl;

R^3 is hydrogen, (C_1-C_5)alkyl, phenyl or p-biphenyl;

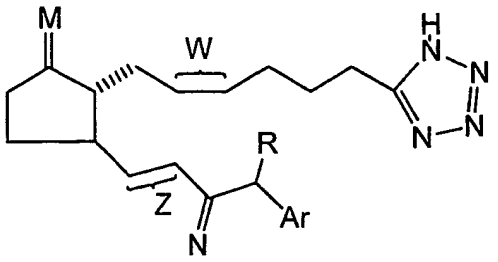
R^4 is COR^5 or SO_2R^5; and

R^5 is phenyl or (C_1-C_5)alkyl.

A preferred group of selective EP_4 receptor agonists of Formula I are those compounds of Formula I wherein Q is 5-tetrazolyl. Particularly preferred compounds within this group include 5-(3-hydroxy-4-phenyl-but-1-enyl)-1-[6-(1H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one and 5-(3-hydroxy-4-phenyl-butyl)-1-[6-(1H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one.

Another preferred group of selective EP_4 receptor agonists of Formula I are those compounds of Formula I wherein Q is COOH. Particularly preferred compounds within this group include 7-[2-(3-hydroxy-4-phenyl-but-1-enyl)-5-oxo-pyrrolidin-1-yl]-heptanoic acid and 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid.

Another preferred group of selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula II:



II

prodrugs thereof or pharmaceutically acceptable salts of said compounds or said prodrugs, wherein:

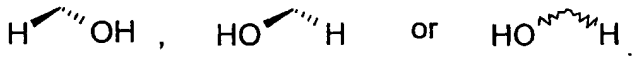
Ar is α- or β-thienyl, 5-phenyl-α- or β-thienyl, 5-lower alkyl-α- or β-thienyl, α- or β-napthyl, tropyl, phenyl, 3,5-dimethylphenyl, 3,4-dimethoxyphenyl, 3,4-methylenedioxyphenyl, 3,4-dichlorophenyl, or mono-substituted phenyl wherein said substituent is bromo, chloro, fluoro, trifluoromethyl, phenyl, lower alkyl, or lower alkoxy;

R is hydrogen or methyl;

W is a single bond or cis double bond;

Z is a single bond or trans double bond; and

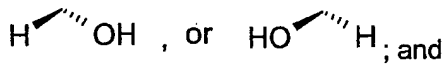
=M and =N are each independently =O,



Another preferred group of selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula II, wherein =M and =N are each =O.

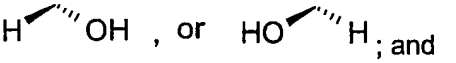
Another preferred group of selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula II wherein

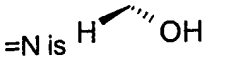
=M is



=N is =O.

Another preferred group of selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula II wherein

=M is 

=N is 

Yet another preferred group of selective EP_4 receptor agonists for use in the methods of the present invention are compounds of Formula II wherein =M is =O; and

=N is  .

DETAILED DESCRIPTION OF THE INVENTION

The term "treating", "treat" or "treatment" as used herein includes preventative (e.g., prophylactic), palliative and curative treatment.

The term "pharmaceutically acceptable" means the carrier, vehicle, diluent, excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the patient.

The expression "prodrug" refers to a compounds that is a drug precursor which, following administration, releases the drug *in vivo* via some chemical or physiological process (e.g., a prodrug on reaching the physiological pH or through enzyme action is converted to the desired drug form). Exemplary prodrugs upon cleavage release the corresponding drug compounds.

The expression "pharmaceutically acceptable salt" refers to nontoxic anionic salts containing anions such as, but not limited to, chloride, bromide, iodide, sulfate, bisulfate, phosphate, acetate, maleate, fumarate, oxalate, lactate, tartrate, citrate, gluconate, methanesulfonate and 4-toluene-sulfonate. The expression also refers to nontoxic cationic salts such as, but not limited to, sodium, potassium, calcium, magnesium, ammonium or protonated benzathine (N,N'-dibenzylethylenediamine), choline, ethanolamine, diethanolamine, ethylenediamine, meglamine (N-methyl-glucamine), benethamine (N-benzylphenethylamine), piperazine and tromethamine (2-amino-2-hydroxymethyl-1,3-propanediol).

The term "selective EP_4 receptor agonist" as used herein is a compound of Formula I or Formula II having a higher binding affinity for the EP_4 receptor than the EP_1, EP_2, and EP_3 receptors. A preferred group of the selective EP_4 receptor agonists are those compounds of Formulae I and II with an IC_{50} at the EP_1, EP_2 and EP_3 receptor at least 10-fold greater than the IC_{50} at the EP_4 receptor subtype. Accordingly, high selectivity or specificity for the EP_4 receptor, compared to other prostaglandin receptors, characterizes the compounds to be used in the methods of the present invention. Also, the receptor selectivity of the compounds to be used in

the methods of the present invention results in the lessening or elimination of undesirable side effects caused by nonselective agents.

The methods of the present invention also include the use of isotopically-labeled compounds, which are identical to those recited in Formula I or Formula II, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of Formula I or Formula II include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Methods of treatment with compounds of Formula I or Formula II, prodrugs thereof, and pharmaceutically acceptable salts of said compounds and said prodrugs, and stereoisomers and diastereomeric mixtures of said compounds, prodrugs and salts, which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labeled compounds of Formula I or Formula II, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds of Formula I or Formula II and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in U.S. Patent No. 4,177,346, U.S. Patent Application Publication US 2001/0047105, published on November 29, 2001 and U.S. Patent No. 3,932,389, by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

The compounds of Formula I or Formula II used in the methods of this invention have asymmetric carbon atoms, and therefore are enantiomers or diastereomers. Diasteromeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known *per se*, for example, by chromatography and/or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixture into a diasteromeric mixture by reaction with an appropriate optically active compound

(e.g., alcohol), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. Enantiomers and diastereomers of the compounds of Formula I or Formula II can also be prepared by utilizing suitable enantiomerically enriched starting materials, or by asymmetric or diastereoselective reactions to introduce asymmetric carbon atoms with the correct stereochemistry. All such isomers, including diastereomers, enantiomers and mixtures thereof are considered as compounds of Formula I or Formula II and can be used in the methods of this invention. Some of the compounds of Formula I or Formula II are acidic, and therefore, can form a salt with a pharmaceutically acceptable cation. All such salts are within the scope of the compounds of Formula I or Formula II, and can be prepared by conventional methods. For example, the salt can be prepared simply by contacting the acidic and basic entities, usually in a stoichiometric ratio, in either an aqueous, non-aqueous or partially aqueous medium, as appropriate. The salts are recovered either by filtration, by precipitation with a non-solvent followed by filtration, by evaporation of the solvent, or, in the case of aqueous solutions, by lyophilization, as appropriate.

The selective EP_4 receptor agonists used in the methods of this invention can be adapted to therapeutic use in animals, e.g., mammals, and particularly humans. The utility of the selective EP_4 receptor agonists used in the methods of the present invention as medical agents in the treatment of liver failure, the loss of patency of the ductus arteriosus, glaucoma or ocular hypertension in animals, e.g., mammals, especially humans, is demonstrated by the activity of those agonists in conventional assays, including the EP_1, EP_2, EP_3, EP_4 receptor binding assay, the cyclic AMP assay, and can be demonstrated by activity in *in vivo* assays, including the liver failure model, all of which are described below. *In vivo* models, such as those described in U.S. Patent Nos. 5,057,621, 5,462,968, and 5,698,598, can be used to demonstrate the ocular hypotensive effect of Formulae I and II compounds. Such assays also provide a means whereby the activities of the selective EP_4 receptor agonists can be compared to each other and with the activities of other known compounds and compositions. The results of these comparisons are useful for determining dosage levels in animals, e.g., mammals, including humans, for the treatment of such diseases.

Administration of a selective EP_4 receptor agonist according to the methods of this invention can be via any available mode that delivers the selective EP_4 receptor

agonist systemically and/or locally (e.g. at the liver, ductus arteriosus, or eyes). These methods include oral routes, parenteral, intraduodenal routes, etc. Generally, the compounds of this invention are administered orally, but parenteral administration (e.g., intravenous, intramuscular, transdermal, subcutaneous, rectal or intramedullar) may be utilized, for example, where oral administration is inappropriate for the target or where the patient is unable to ingest the drug.

The methods of this invention are used for the treatment of liver failure, loss of patency of the ductus arteriosus, glaucoma, or ocular hypertension and can be carried out by either systemic or local application (e.g., to the ductus arteriosus, liver, or eyes) of the selective EP_4 receptor agonists. The selective EP_4 receptor agonists useful in the methods of the present invention are applied to the sites of the ductus arteriosus or liver, for example, either by injection of the compound in a suitable solvent, or in cases of open surgery, by local application thereto of the compound in a suitable vehicle, carrier or diluent. For administration to the eye, an ophthalmic preparation such as a gel, ointment, solution or suspension can be employed.

In any event, the amount and timing of the compound administered will be dependent on the patient being treated, on the severity of the affliction, on the manner of administration and on the judgment of the prescribing physician. Thus, because of patient to patient variability, the dosages given herein are a guideline and the physician may titrate doses of the drug compound to achieve the treatment (e.g., treat liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension) that the physician considers appropriate for the patient. In considering the degree of treatment desired, the physician must balance a variety of factors such as age of the patient, body weight of the patient, symptom, presence of preexisting disease, desired therapeutic effect, the route of administration, and the duration of the treatment etc. In the human adult, the doses per person per dose are generally 1 μg to 100 mg, by oral administration, from once up to several times per day, and from 0.1 μg to 10 mg, by parenteral administration (preferably intravenously) from once up to several times per day, or by continuous administration for from 1 to 24 hours per day by intravenous infusion. For the treatment of neonates the dosage will have to be adjusted accordingly due to the patient's young age and low body weight. In general, in the methods of the present invention an amount of the selective EP_4 receptor agonist (compound of Formulae I and II) is used that is sufficient to treat liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension. As

the doses to be administered depend upon various conditions, there are cases in which doses lower or higher than the ranges specified above can be used.

The selective EP_4 receptor agonist compounds used in the methods of this invention are generally administered in the form of a pharmaceutical composition comprising at least one of the compounds of this invention together with a pharmaceutically acceptable vehicle or diluent. Thus, the selective EP_4 receptor agonist compound can be administered individually in any conventional form, such as oral, intranasal, parenteral, rectal or transdermal dosage form.

For oral administration the pharmaceutical composition can take the form of solutions, suspensions, tablets, pills, capsules, powders, and the like. Tablets containing various excipients such as sodium citrate, calcium carbonate and calcium phosphate are employed along with various disintegrants such as starch, preferably potato or tapioca starch, and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the compositions of this invention can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

The compounds can also be administered orally in solid solution with lipids such as cholesterol acetate. The inclusion of lipid in the formulation markedly increases absorption of the compound or analog. Preparation of such formulations is described in detail in Rudel, U.S. Patent No. 3,828,106.

For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions of the corresponding water-soluble salts. Such aqueous solutions may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In this

connection, the sterile aqueous media employed are all readily obtainable by standard techniques well known to those skilled in the art.

Compositions to be administered intravenously or by injection can be prepared as solutions of the compound in, for example, an isotonic aqueous solution, an alcohol solution, an ethanol-saline solution, or an ethanol-dextrose solution. Ethanol can be added to the solution to increase solubility and other additives such as methylparaben or other ingredients such as fillers, colorings, flavorings, diluents and the like can be included. The composition can also be administered as a suspension of the compound or analog in aqueous or non-aqueous media.

Among the preferred formulations for administration intravenously or by injection are complexes of the active ingredient with α-cyclodextrin. Preparation of complexes of compounds and analogs with α-cyclodextrin clathrates are described in detail in Hayashi et al., U.S. Patent No. 4,054,736. Complexes wherein the ratio of α-cyclodextrin to a compound of this invention is 97:3 are especially preferred.

For purposes of transdermal (e.g.,topical) administration, dilute sterile, aqueous or partially aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared.

For purposes of ophthalmic administration, an aqueous solution of the compound of Formula I or Formula II is generally preferred (typical concentration range is 0.001 to approximately 1% weight/volume). The aqueous solution can then be administered by instilling drops of the solution to the patient's eyes (usually 1 to 2 drops administered 1 to 4 times a day). For compounds of Formula I or Formula II with less water solubility, an aqueous suspension may be preferred. Other ophthalmic compositions known in the art, such as viscous or semi-viscous gels, or other types of solid or semi-solid compositions containing compounds of Formula I or Formula II may be employed.

The ophthalmic composition may also contain a preservative such as benzalkonium chloride, chlorobutanol, edetate disodium, phenylethyl alcohol, phenylmercuric acetate, phenyl mercuric nitrate, methyl paraben, propyl paraben, polyquaternium-1, sorbic acid, thimerosal, or other known preservatives (typical concentration range of the preservative is 0.001 to 1.0% weight/volume). A surfactant, such as Tween 80, can also be used in the ophthalmic composition. Various vehicles, such as polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose cyclodextrin

and water can be used for the ophthalmic composition. The tonicity of the ophthalmic composition can be adjusted using a tonicity adjustor such as sodium chloride, potassium chloride, mannitol or glycerin. The ophthalmic composition can be buffered, preferably to a range of 4.5 to 8.0, using buffers such as acetate buffers, citrate buffers, phosphate buffers and borate buffers. The pH of the ophthalmic composition can be adjusted, preferably to a range between 4.5 to 8.0 using an appropriate acid or base. Antioxidants, such as sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene can also be used in the ophthalmic composition.

Methods of preparing various pharmaceutical compositions with a certain amount of active ingredient are known, or will be apparent in light of this disclosure, to those skilled in the art. For examples of methods of preparing pharmaceutical compositions, see Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa., 19th Edition (1995). Thus, as described above, the compounds of this invention may be administered to the patients in any of the known formulations or modes of administration.

Combination therapy can also be used in the methods of the present invention for th treatment of glaucoma or ocular hypertension. For the treatment of glaucoma or ocular hypertension, the selective EP_4 receptor agonists of Formula I or Formula II can be combine with other medicaments known to be useful for the treatment of glaucoma (anti-glaucoma agents), such as β-adrenergic blocking agents, carbonic anhydrase inhibitors, miotics and sympathomimetics. For example, β -adrenergic agents such as betaxolol, including its hydrochloride salt, and timolol, including its maleate salt can be combined with the selective EP_4 receptor agonists of Formula I or Formula II. Some examples of specific carbonic anhydrase inhibitors that can be used in combination with the selective EP_4 receptor agonis of Formula I or Formula II include brinzolamide, dichlorphenamide, and dorzolamide, including its hydrochloride salt. Miotics, such as demecarium bromide, can also be used in combination with the selective EP_4 receptor agonists of Formula I or Formula II. Sympathomimetics, such as brimonidine, including its tartrate salt, pheniramine, including it maleate salt, and phenylephrine, including its hydrochloride salt, can be used in combinatio with the selective EP_4 receptor agonists of Formula I or Formula II.

Advantageously, the present invention also provides kits for use by a consumer to treat liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension. The kits comprise a) a pharmaceutical composition comprising

a selective EP_4 receptor agonist (compound of Formula I or II); b) instructions describing methods of using the pharmaceutical compositions to treat liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension; and c) a container. For methods of treating glaucoma or ocular hypertension the kit may also contain an anti-glaucoma agent as described above.

A "kit" as used in the instant application includes a container for containing the pharmaceutical compositions and may also include divided containers such as a divided bottle or a divided foil packet. The container can be in any conventional shape or form as known in the art which is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle, which is in turn contained within a box.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably, the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It may be desirable to provide a written memory aid, where the written memory aid is of the type containing information and/or instructions for the physician, pharmacist or other health care provider, or patient, e.g., in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested or a card which contains the same type of information. Another example of such a memory aid is a calendar printed on the card e.g., as follows "First Week, Monday, Tuesday," . . . etc . . . . "Second Week, Monday, Tuesday, . . ." etc. Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day.

Another specific embodiment of a kit is a dispenser designed to dispense the daily doses one at a time. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

The documents cited herein, including any patents and patent applications, are hereby incorporated by reference.

EXPERIMENTAL SECTION

In vitro assays

The compounds of Formula I or II, which are useful in the methods of the present invention, bind to the prostaglandin E_2 type 4 receptor (EP_4 receptor). The full-length coding sequence for the human EP_1 receptor is made in accordance with the procedure in Funk et al., Journal of Biological Chemistry, **1993**, *268*, 26767-26772. The full-length rat EP_2 receptor is made in accordance with the procedure in Nemoto et al., *Prostaglandins and other Lipid Mediators*, **1997**, *54*, 713-725. The full-length coding sequence for the human EP_3 receptor is made in accordance with the procedure in Regan et al., British Journal of Pharmacology, **1994**, *112*, 377-385. The full-length coding sequence for the rat EP_4 receptor is made in accordance with the procedure in Sando et al., Biochem. Biophys. Res. Comm. **1994**, *200*, 1329-1333. These full-length receptors are used to prepare 293S cells expressing the human EP_1, rat EP_2, human EP_3 or rat EP_4 receptors.

Human EP_1, Rat EP_2, Human EP_3, Rat EP_4 Receptor Binding Assay

The full-length receptors described above are used to prepare 293S cells expressing the EP_1, EP_2, EP_3, and EP_4 receptors.

293S cells expressing either the human EP_1, rat EP_2, human EP_3 or rat EP_4 prostaglandin E_2 receptors are generated according to methods known to those skilled in the art. Typically, PCR (polymerase chain reaction) primers corresponding to the 5' and 3' ends of the published full length receptor are made according to the well known methods disclosed above and are used in an RT-PCR (reverse transcriptase-polymerase chain reaction) reaction using the total RNA from human kidney (for EP_1), rat kidney (for EP_2), human lung (for EP_3), or rat kidney (EP_4) as a source. PCR products are cloned by the TA overhang method into pCR2.1 (Invitrogen Corporation, Carlsbad, CA) and identity of the cloned receptor is confirmed by DNA sequencing. For expression of the rat EP_2 receptor, the confirmed cDNA is subcloned into the mammalian expression vector PURpCI, a vector generated by subcloning the selectable marker for puromycin resistance into the mammalian expression vector pCI (Promega, Madison, WI)

293S cells are transfected with either the cloned human EP_1 or EP_3 receptor in pcDNA3 by electroporation. Stable cell lines expressing either the human EP_1 or EP_3 receptor are established following selection of transfected cells with G418. 293S cells are transfected with the cloned rat EP_2 receptor in PURpCi by lipid mediated transfection. Stable cell lines expressing the rat EP_2 receptor are established following selection of transfected cells with puromycin. 293S cells are transfected with the cloned rat EP_4 receptor in pcDNA3 by lipid mediated transfection. Stable cell lines expressing the rat EP_4 receptor are established following selection of transfected cells with Geneticin® (Invitrogen, Carlsbad, CA).

Clonal cell lines expressing the maximal number of receptors are chosen following a whole cell ^{3}H-PGE_2 binding assay using unlabeled PGE_2 as a competitor.

Membrane Preparation: All operations are performed at 4 °C. Transfected cells expressing either prostaglandin E_2 type 1, type 2, type 3, or type 4 (EP_1, EP_2, EP_3, or EP_4, respectively) receptors are harvested and suspended to 2 million cells per ml in Buffer A [50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 1 mM

Pefabloc peptide, (Boehringer Mannheim Corp., Indianapolis, IN), 10 uM Phosporamidon peptide, (Sigma, St. Louis, MO), 1 uM pepstatin A peptide, (Sigma, St. Louis, MO), 10 uM elastatinal peptide, (Sigma, St. Louis, MO), 100 uM antipain peptide, (Sigma, St. Louis, MO)]. The cells are lysed by sonification with a Branson Sonifier (Branson Ultrasonics Corporation, Danbury, CT) in 2 fifteen-second bursts. Unlysed cells and debris are removed by centrifugation at 100 x g for 10 min. Membranes are then harvested by centrifugation at 45,000 x g for 30 minutes. Pelleted membranes are resuspended to 3-10 mg protein per ml, protein concentration being determined of the method of Bradford [Bradford, M., Anal. Biochem. **1976**, *72*, 248]. Resuspended membranes are then stored frozen at -80 °C until use.

Binding Assay: Frozen membranes prepared as above are thawed and diluted to 1 mg protein per ml in Buffer A above. 100 µl of the cell membrane preparation is combined with 5 µl of a solution of test compound of Formula I or II (diluted in DMSO to a concentration 40 times the desired final concentration) and 95 µl of 3 nM ^{3}H-prostaglandin E_2 (Amersham, Arlington Heights, IL) in Buffer A. The mixture (200 µL total volume) is incubated for 1 hour at 25°C. The membranes are then recovered by filtration through type GF/C glass fiber filters (Wallac, Gaithersburg, MD) using a Tomtec harvester (Tomtec, Orange, CT). The membranes with bound ^{3}H-prostaglandin E_2 are trapped by the filter, while the buffer and unbound ^{3}H-prostaglandin E_2 pass through the filter into waste. Each sample is then washed 3 times with 3 ml of [50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA]. The filters are then dried, by heating in a microwave oven. To determine the amount of ^{3}H-prostaglandin bound to the membranes, the dried filters are placed into plastic bags with scintillation fluid and counted in a LKB 1205 Betaplate reader (Wallac, Gaithersburg, MD). IC_{50}s are determined from the concentration of test compound required to displace 50% of the specifically bound ^{3}H-prostaglandin E_2.

Determination of cyclic AMP Elevation in 293S Cell Lines Stably Overexpressing Recombinant Rat EP_4 Receptors Assay

cDNA representing the complete open reading frame of the rat EP_4 receptor is generated by reverse transcriptase polymerase chain reaction using oligonucleotide primers based on published sequences. The full length coding

sequence for the rat EP_4 receptor is made in accordance with the procedure in Sando et al., Biochem. Biophys. Res. Comm. **1994**, *200*, 1329-1333, and RNA from rat kidney (EP_4) as templates. 293S cells are transfected with the cloned rat EP_4 receptor in pcDNA3 by lipid mediated transfection. Stable cell lines expressing the rat EP_4 receptor are established following selection of transfected cells with Geneticin® (Invitrogen Corporation, Carlsbad, CA).

Clonal cell lines expressing the maximal number of receptors are chosen following a whole cell ^{3}H-PGE_2 binding assay using unlabeled PGE_2 as a competitor. Transfectants demonstrating high levels of specific [^{3}H]PGE_2 binding are further characterized by Scatchard analysis to determine B_{max} and K_ds for PGE_2. The lines selected for compound screening have approximately 256,400 receptors per cell and a K_d = 2.9 nm for PGE_2 (EP_4). Constitutive expression of the receptor in parental 293-S cells is negligible. A stable cell line containing the rat EP_4 receptor is grown in Dulbecco's Mosified Eagle Medium/F12 (DMEM/F12) containing 10% fetal bovine serum and G418 (500 μg/ml) to 80% confluency.

cAMP responses in the 293-S/EP_4 lines are determined by detaching cells from culture flasks in 1 ml of calcium (Ca++) and magnesium (Mg++) deficient phosphate buffered saline (PBS) via vigorous pounding and then rinsing the cells with calcium (Ca++) and magnesium (Mg++) deficient phosphate buffered saline (PBS). The cells are resuspended in MEM (Minimum Essential Medium), 1% BSA (bovine serum albumin), 50 mM HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) at 37°C. The cell suspension is counted on a hemacytometer and diluted by adding MEM (Minimum Essential Medium) to a final concentration of 1 x 10^6 cells/ml, and adding 3-isobutyl-1-methylxanthine (IBMX) to a final concentration of 1mM. 200 microliters of cell suspension is immediately aliquoted into individual tubes and incubated for 10 minutes, uncovered, at 37 °C, 5% CO_2, 95% relative humidity. The compound of Formula I or II to be tested in either dimethylsulfoxide (DMSO) or ethanol is then added to cells at 1:100 dilutions such that the final DMSO or ethanol concentration is 1%. Typically, the cells are treated with 6-8 different concentrations (in 1 log increments, such as those described below) of the compound of Formula I or II. Typical concentrations of the compound of Formula I or II in this assay are between 10^{-5}M to 10^{-10}M. For example, a six point compound dose response assay tests the compound of Formula I or II at concentrations of 10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M and 10^{-10}M. Immediately after

adding the test compound, the tubes are covered, mixed by inverting two times, and incubated at 37 °C for 12 minutes. Samples are then lysed by incubation at 100 °C for 10 minutes and immediately cooled on ice for 5 minutes to approximately 4°C. Cellular debris is pelleted by centrifugation at 3500 x g for 5 minutes at approximately 4°C, and cleared lysates are transferred to fresh tubes. cAMP concentrations are determined using a commercially available ^{125}I-cAMP radioimmunoassay (RIA) kit (NEK-033, Perkin-Elmer Life Sciences, Inc., Boston, MA). The cleared lysates are diluted 1:100 in cAMP RIA assay buffer (included in kit) and centrifuged again. 50 microliters of the resulting supernatant is transferred to a 12 x 75 mm glass tube and data is collected by scintillation counting using a Wallac Cobra II Gamma Counter (Perkin-Elmer Wallac, Inc., Gaithersburg, MD). EC_{50} calculations are performed on a calculator using linear regression analysis on the linear portion of the dose response curves or using Data Fitter.

In vivo assays

The selective EP_4 receptor agonists of Formula I or Formula II can be evaluated in various *in vivo* liver failure models known in the art, such as an *in vivo* rat liver failure model (Kazuhiro, Kasai. et al., Gastroenterology **2001**, 120 (Suppl. 1), A-541).

In vivo Acute Liver Injury Model

Methods: Acute liver failure in rats can be induced by intraperitoneal injection of one of carbon tetrachloride (CCl_4, 1 mg/kg), dimethylnitrosamine (DMN, 50 mg/kg), D-galactosamine (D-gal, 1 g/kg), or D-galactosamine with lipopolysaccharide (LPS), (D-gal, 1 g/kg; LPS 100 μg/kg). Immediately following the intaperitoneal injection of carbon tetrachloride, dimethylnitrosamine, D-galactosamine, or D-galactosamine with lipopolysaccharide, the test compound of Formula I or II or saline (as control) is administered. The test compound (a selective EP_4 receptor agonist of Formula I or II) can be administered at various doses such as 0.01, 0.05, 0.1 or 0.2 mg/kg. 24 hours after administration of the test compound of Formula I or II, the liver can be removed for histology and serum can be obtained for determination of total bilirubin (T-bil), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Massive hepatic necrosis with marked elevations in the levels of T-bil, AST, and ALT was observed in the saline treated control group. The effectiveness of the test compound

in the above models can be determined by comparison of histology and serum results obtained for the animals treated with the test compound with the corresponding results from the saline control group.

EXAMPLES

The examples presented herein are intended to illustrate particular embodiments of the invention, and are not intended to limit the specification or the claims in any manner.

EXAMPLES 1-10

The *in vitro* Human EP_1, Rat EP_2, Human EP_3, Rat EP_4 Receptor Binding Assay and the Determination of cyclic AMP Elevation in 293S Cell Lines Stably Overexpressing Recombinant Rat EP_4 Receptors Assay, described hereinabove, were used to evaluate the following compounds. The compounds used in Examples 1-8 and 10 were prepared as described in U.S. Patent Application Publication US 2001/0047105, published on November 29, 2001.

Example 1

7-{2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid, prepared according to the procedure for Example 1 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 22 nm (rat EP_4) and >3200 nm (rat EP_2, human EP_1, EP_3) in the binding assay, and an EC_{50} of 8.8 nm in the cAMP (rat EP_4) elevation assay.

Example 2

7-{2S-[3R-hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid, prepared according to the procedure for Example 2 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 21 nm (rat EP_4), 2760 nm (rat EP_2), and >3200 nm (human EP_1, EP_3), in the binding assay, and an EC_{50} of 13.2 nm in the cAMP (rat EP_4) elevation assay.

Example 3

5S-[4-(3-Chloro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 3 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 38 nm (rat EP_4), 2370 nm (rat EP_2), and >3200 nm (human EP_1, EP_3), in the binding assay, and an EC_{50} of 33.1 nm in the cAMP (rat EP_4) elevation assay.

Example 4

5S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 4 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 33 nm (rat EP_4), and >3200 nm (rat EP_2, human EP_1, EP_3), in the binding assay, and an EC_{50} of 70.2 nm in the cAMP (rat EP_4) elevation assay.

Example 5

5-[4-(4-Fluoro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 5 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 508 nm (rat EP_4), and >3200 nm (rat EP_2, human EP_1, EP_3), in the binding assay.

Example 6

5-(4-Biphenyl-3-yl-3-hydroxy-butyl)-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 6 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 50 nm (rat EP_4), 3050 nm (rat EP_2) and >3200 nm (human EP_1, EP_3), in the binding assay, and an EC_{50} of 175 nm in the cAMP (rat EP_4) elevation assay.

Example 7

5-[4-(3-Fluoro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 7 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 96 nm (rat EP_4), and >3200 nm (rat EP_2), in the binding assay, and an EC_{50} of 200 nm in the cAMP (rat EP_4) elevation assay.

Example 8

5S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, prepared according to the procedure for Example 8 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 28 nm (rat EP_4), and >3200 nm (rat EP_2), in the binding assay, and an EC_{50} of 24.6 nm in the cAMP (rat EP_4) elevation assay.

Example 9

7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid was found to have IC_{50}s of 54 nm (rat EP_4), and >3200 nm (rat EP_2, human EP_1, EP_3), in the binding assay, and an EC_{50} of 32.5 nm in the cAMP (rat EP_4) elevation assay.

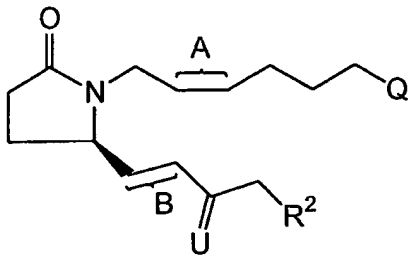
Example 10

7-{2S-[3-Hydroxy-4-(3-phenoxy-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid, prepared according to the procedure for Example 10 in U.S. Patent Application Publication US 2001/0047105, was found to have IC_{50}s of 536 nm (rat EP_4), and >3200 nm (rat EP_2), in the binding assay.

CLAIMS

What is claimed is:

1. A method of treating liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension, comprising administering to a patient in need thereof a compound of Formula I:



I

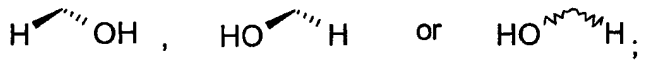
a prodrug thereof, or a pharmaceutically acceptable salt of the compound or prodrug, wherein:

Q is $COOR^3$, $CONHR^4$ or tetrazol-5-yl;

A is a single or cis double bond;

B is a single or trans double bond;

=U is =O,



R^2 is α-thienyl, phenyl, phenoxy, monosubstituted phenyl or monosubstituted phenoxy, said substituents being selected from the group consisting of chloro, fluoro, phenyl, methoxy, trifluoromethyl and $(C_1\text{-}C_3)$alkyl;

R^3 is hydrogen, $(C_1\text{-}C_5)$alkyl, phenyl or p-biphenyl;

R^4 is COR^5 or SO_2R^5; and

R^5 is phenyl or $(C_1\text{-}C_5)$alkyl.

2. A method of claim 1, wherein Q is 5-tetrazolyl.

3. A method of claim 2, wherein the compound of Formula I is 5-(3-Hydroxy-4-phenyl-but-1-enyl)-1-[6-(1H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, 5-(3-Hydroxy-4-phenyl-butyl)-1-[6-(1H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, 5S-[4-(3-Chloro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one,

5S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one,

5S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one,

5-[4-(4-Fluoro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one,

5-(4-Biphenyl-3-yl-3-hydroxy-butyl)-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one, or

5-[4-(3-Fluoro-phenyl)-3-hydroxy-butyl]-1-[6-(2H-tetrazol-5-yl)-hexyl]-pyrrolidin-2-one.

4. A method of claim 1, wherein Q is COOH.

5. A method of claim 4, wherein the compound of Formula I is

7-(2-(3-Hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid,

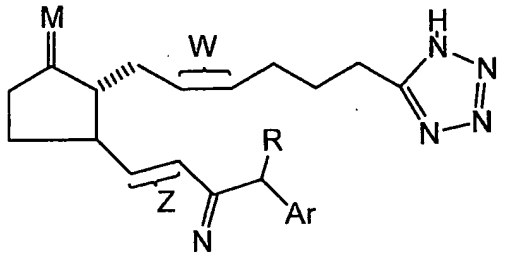
7-[2-(3-Hydroxy-4-phenyl-but-1-enyl)-5-oxo-pyrrolidin-1-yl]-heptanoic acid,

7-{2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid,

7-{2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid, or

7-{2S-[3-Hydroxy-4-(3-phenoxy-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl}-heptanoic acid.

6. A method of treating liver failure, loss of patency of the ductus arteriosus, glaucoma or ocular hypertension, comprising administering to a patient in need thereof a compound of Formula II:



II

a prodrug thereof, or a pharmaceutically acceptable salt of the compound or prodrug, wherein:

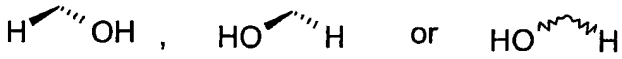
Ar is α- or β-thienyl, 5-phenyl-α- or β-thienyl, 5-lower alkyl-α- or β-thienyl, α- or β-napthyl, tropyl, phenyl, 3,5-dimethylphenyl, 3,4-dimethoxyphenyl, 3,4-methylenedioxyphenyl, 3,4-dichlorophenyl, or mono-substituted phenyl wherein said substituent is bromo, chloro, fluoro, trifluoromethyl, phenyl, lower alkyl, or lower alkoxy;

R is hydrogen or methyl;

W is a single bond or cis double bond;

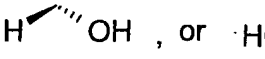
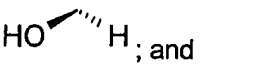
Z is a single bond or trans double bond; and

=M and =N are each independently =O,

 , or

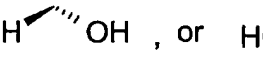
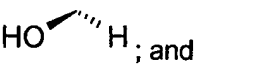
7. A method of claim 6, wherein =M and =N are each =O.

8. A method of claim 6, wherein =M is

 , or  ; and

=N is =O.

9. A method of claim 6, wherein =M is

 , or  ; and

=N is  .

10. A method of claim 6, wherein =M is =O; and

=N is  .

11. The method of claim 1, wherein the method is the treatment of liver failure.

12. The method of claim 1, wherein the method is the treatment of the loss of patency of the ductus arteriosus.

13. The method of claim 1, wherein the method is the treatment of glaucoma or ocular hypertension.

14. The method of claim 6, wherein the method is the treatment of liver failure.

15. The method of claim 6, wherein the method is the treatment of the loss of patency of the ductus arteriosus, glaucoma or ocular hypertension.

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/IB 03/00955

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/40 A61K31/41

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01 46140 A (KE HUAZHU ;PFIZER PROD INC (US); CAMERON KIMBERLY O KEEFE (US); LE) 28 June 2001 (2001-06-28) page 3, line 29 -page 5, line 20	1-5, 11-13
Y	US 3 932 389 A (JOHNSON MICHAEL R ET AL) 13 January 1976 (1976-01-13) column 17, line 20 - line 23; claims 3-9	6-10,14, 15
Y	EP 1 097 922 A (ONO PHARMACEUTICAL CO) 9 May 2001 (2001-05-09) paragraph '0047!	1-11,14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2003	10/06/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Loher, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	KASAI K ET AL: "A novel prostaglandin E receptor subtype agonist, ONO-4819, attenuates acute experimental liver injury in rats" HEPATOLOGY RESEARCH 2001 IRELAND, vol. 21, no. 3, 2001, pages 252-260, XP002242730 ISSN: 1386-6346 Results page 253, left-hand column, paragraph 2; figure 1 ---	1-11,14
Y	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2002 TANIGUCHI TAKANOBU ET AL: "EP4 agonist, AE1, abrogates indomethasin-induced Ductus Arteriosus constriction in utero." Database accession no. PREV200200254678 XP002242731 See especially the statement made by the title & JAPANESE JOURNAL OF PHARMACOLOGY, vol. 88, no. Supplement 1, 2002, page 270P 75th Annual Meeting of the Japanese Pharmacological Society;Kumamoto, Japan; March 13-15, 2002, 2002 ISSN: 0021-5198 ---	1-10,12, 15
Y	WO 00 38667 A (ALCON LAB INC ;KLIMKO PETER G (US); SHARIF NAJAM A (US); GRIFFIN B) 6 July 2000 (2000-07-06) page 10, line 5 - line 10 ---	1-10,13, 15
X,P	WO 03 008377 A (HOFFMANN LA ROCHE) 30 January 2003 (2003-01-30) page 14, line 17 page 34, line 4 page 34, line 24 see especially formula I -----	1,2,4,11

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Int onal application No.
PCT/IB 03/00955

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/IB 03/00955

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0146140 A	28-06-2001	AU 1293101 A	03-07-2001
		AU 7239300 A	28-06-2001
		BG 106882 A	28-02-2003
		BR 0016560 A	10-09-2002
		CA 2329678 A1	22-06-2001
		CN 1413190 T	23-04-2003
		EP 1110949 A1	27-06-2001
		HU 0005001 A2	28-12-2001
		WO 0146140 A1	28-06-2001
		JP 2001181210 A	03-07-2001
		NO 20022925 A	18-06-2002
		TR 200201643 T2	21-11-2002
		US 2001047105 A1	29-11-2001
		US 2002040149 A1	04-04-2002
US 3932389 A	13-01-1976	AR 222623 A1	15-06-1981
		AU 8736375 A	02-06-1977
		BE 836563 A1	11-06-1976
		CA 1059122 A1	24-07-1979
		CA 1053684 A2	01-05-1979
		CH 614434 A5	30-11-1979
		CH 612188 A5	13-07-1979
		CS 191995 B2	31-07-1979
		CS 191996 B2	31-07-1979
		CS 191960 B2	31-07-1979
		DD 129644 A5	01-02-1978
		DD 129648 A5	01-02-1978
		DK 560075 A	12-06-1976
		ES 443422 A1	16-04-1977
		FI 753474 A ,B,	12-06-1976
		FI 803527 A	11-11-1980
		FR 2293927 A1	09-07-1976
		GB 1515415 A	21-06-1978
		GB 1515414 A	21-06-1978
		GR 58521 A1	29-10-1977
		HU 175278 B	28-06-1980
		IE 42938 B1	19-11-1980
		IE 42937 B1	19-11-1980
		IL 48637 A	30-12-1979
		JP 1068610 C	23-10-1981
		JP 52116471 A	29-09-1977
		JP 56008035 B	20-02-1981
		JP 970103 C	31-08-1979
		JP 51086465 A	29-07-1976
		JP 54001711 B	27-01-1979
		LU 73996 A1	11-11-1976
		MX 3660 E	15-04-1981
		NL 7514447 A ,B,	15-06-1976
		NO 754174 A ,B,	14-06-1976
		NZ 179503 A	10-07-1978
		NZ 183061 A	10-07-1978
		SE 415759 B	27-10-1980
		SE 7907780 A	19-09-1979
		US 4035360 A	12-07-1977
		YU 313775 A1	30-06-1982
		ZA 7507754 A	24-11-1976
EP 1097922 A	09-05-2001	AU 4651899 A	07-02-2000

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/IB 03/00955

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1097922 A		BR 9912813 A	02-05-2001
		CA 2336952 A1	27-01-2000
		EP 1097922 A1	09-05-2001
		HU 0204170 A2	28-04-2003
		JP 3174563 B2	11-06-2001
		NO 20010213 A	15-03-2001
		US 6462081 B1	08-10-2002
		CN 1312796 T	12-09-2001
		WO 0003980 A1	27-01-2000
		JP 2001089444 A	03-04-2001
		TR 200100623 T2	21-06-2001
WO 0038667 A	06-07-2000	AU 2211700 A	31-07-2000
		WO 0038667 A2	06-07-2000
		US 6545045 B1	08-04-2003
WO 03008377 A	30-01-2003	WO 03008377 A1	30-01-2003